Molecular Dimensions

Soluble Proteins

The BCS Screen HT-96 MD1–105

A 96 condition protein crystallization screen incorporating PEG smears[1] for broader chemical space coverage.

MD1-105 is presented as 96 x 1 mL conditions.

Features of The BCS Screen:

- Contains defined PEG Smears – efficient screening of chemical space.
- Wide PEG coverage.
- Screen against multiple additives. Contains multiple salts/additives shown to promote crystallization.
- Proven crystallization successes with multiple proteins.

Introduction

The Basic **C**hemical **S**pace (**BCS) Screen** developed at the Structural Genomics Consortium (SGC), Oxford, UK, has been formulated to include PEG smears - enabling a broader search of chemical space than just using individual PEGS.

The benefit of using PEG smears is that it reduces the number of PEG variables while maintaining a large coverage of PEG space. The PEGs in **The BCS Screen** are grouped by molecular weight and mixed to create four PEG smears (available to purchase separately). The smears cover a broader range of chemical space while reducing the number of PEG variables.

The BCS Screen was tested for crystallization potency using a set of 191 proteins from projects at the Structural Genomics Consortium (SGC) based in Oxford, UK. The success rates of the smear-based screen were similar to the combined success rates of the other four in-house screens exploited regularly at the SGC. In particular, the ability of the smear-based screen promoted crystal growth of several proteins that had been difficult to crystallize (Figure 1) using the commercial sets.

The BCS Screen: The first 24 conditions in The BCS Screen form a grid screen of the four PEG Smears against pH (range 4.5-9.5). The remaining 72 conditions consist of a sparse matrix screen in which the precipitant is always one of the PEG smears but with a variety of additives and buffers. The additives include some of the more unusual crystal-promoting agents such as Rubidium chloride (also useful for phasing) and ammonium nitrate. More common additives including Sodium Bromide (useful for phasing) and glycerol are also used in the screen. In addition, multiple salts/additives are implemented, which have been shown also to promote crystallization.

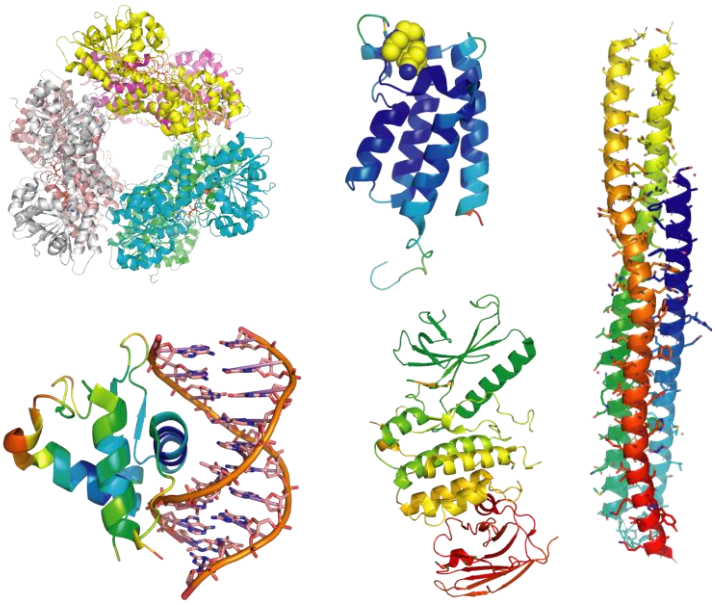

*Figure 1. Examples of crystal structures obtained from using **The BCS Screen** at the SGC. For example, single-domain proteins, protein-protein complex and protein-DNA complex.*

The BCS Screen is formulated in collaboration with, Apirat Chaikuad, Elizabeth MacLean, Stefan Knapp and Frank von Delft at the Structural Genomics Consortium, Oxford, UK.

[1] PEG smears, originally suggested by Janet Newman (ActaD 2005)

PEG Smear compositions used in The BCS Screen

Eleven PEGs were selected from those commonly used in most commercial screens (Figure 2) to provide molecular weight (MW) coverage from 400 to 10k Da, namely PEG 400, 550 MME, 600, 1K, 2K, 3350, 4K, 5KMME, 6K, 8K and 10K.
These eleven PEGs were then divided into three classes based on molecular weight, low (≤1k Da), medium (>1k-5k Da), and high (≥6k Da).

The four smears in The BCS Screen consist of:

PEG Smear	Composition	Catalogue Number (100 mL)	Catalogue Number (250 mL)
50% v/v PEG Smear Low	12.5%v/v PEG 400 12.5%v/v PEG 500 MME 12.5%v/v PEG 600 12.5%w/v PEG 1000	MD2-100-258	MD2-250-258
50% v/v PEG Smear Medium	12.5%w/v PEG 3350 12.5%w/v PEG 4000 12.5%w/v PEG 2000 12.5%w/v PEG 5000 MME	MD2-100-259	MD2-250-259
50% v/v PEG Smear High	16.67%w/v PEG 8000 16.67%w/v PEG 10000 16.67%w/v PEG 6000	MD2-100-260	MD2-250-260
50% v/v PEG Smear Broad*	4.55%v/v PEG 400 4.55%v/v PEG 500 MME 4.55%v/v PEG 600 4.55%w/v PEG 1000 4.55%w/v PEG 2000 4.55%w/v PEG 3350 4.55%w/v PEG 4000 4.55%w/v PEG 5000 MME 4.55%w/v PEG 6000 4.55%w/v PEG 8000 4.55%w/v PEG 10000	MD2-100-261	MD2-250-261

***** The Broad Smear is similar to the originally described smear (Newman et al., 2005), which did not however contain any methylated polymers.

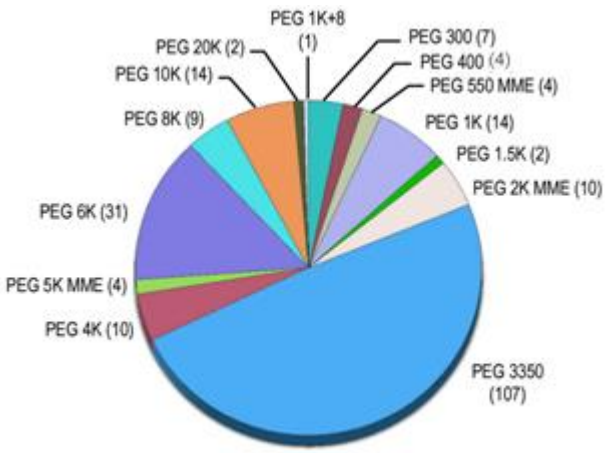

Figure 2. Bias in PEG molecular weights in four of the most common sparse matrix screens. Taken from Chaikuad *et al* (2015).

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Formulation Notes:

The BCS Screen reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Final pH may vary from that specified on the datasheet. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.
Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Enquiries regarding **The BCS Screen** formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at www.moleculardimensions.com

Hints & Tips

The BCS Screen conditions are not cryoprotected, so cryoprotection will have to be carried out. For this we recommend CryoProtX (MD1-61).

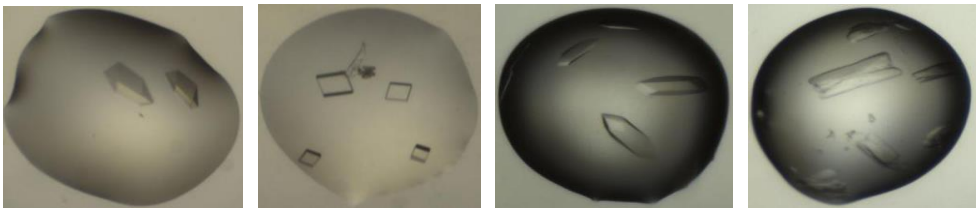
To optimize PEG Smears we recommend the following: treat the PEG smears as 'normal PEG' and just vary the concentration, or deconvolute the effects of the individual PEGs as described in Chaikuad *et al* (2015).

The screen has been predominantly tested with soluble proteins, but this does not mean it cannot work for membrane proteins.

Publications resulting from crystals grown using The BCS screen include:

Chaikuad, A., Knapp, S. & von Delft, F. (2015). Acta Cryst. D71, 1627-1639.
Froese DS *et al*, (2010) J Biol Chem. Dec 3;285(49):38204-13.
Filippakopoulos P *et al*, (2012) Cell. Mar 30;149(1):214-31.
Chaikuad A *et al*, (2011) Proc Natl Acad Sci U S A, Dec 27; 108(52):21028-33.
Chaikuad A *et al*, (2014) Nat Chem Biol. Oct;10(10):853-60.
Kovackova S *et al*,(2015) J Med Chem. Apr 23;58(8):3393-410
Egger S *et al*, (2012) J Biol Chem. Jan 13;287(3):2119-29
Chaikuad A *et al*, (2016) J Med Chem. Feb 25;59(4):1648-53
Chen P *et al*, (2016) J Med Chem. Feb 25;59(4):1410-24
Drouin L *et al*, (2015) J Med Chem. Mar 12;58(5):2553-9

*Protein crystals grown using **The BCS Screen**. Images courtesy of the SGC, Oxford.*



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Re-Ordering details:

Catalogue Description	Pack size	Catalogue Code
The BCS Screen	96 x 10 mL	MD1-104
The BCS Eco Screen	96 x 10 mL	MD1-104-ECO
The BCS Screen HT-96	96 x 1 mL	MD1-105
The BCS Screen HT-96 Eco Screen	96 x 1 mL	MD1-105-ECO
The BCS Screen FX-96	96 x 100 µL	MD1-105-FX
Single Reagents		
The BCS Screen	100 mL	MDSR-104-tube number
The BCS Screen HT-96	100 mL	MDSR-105-well number
50% v/v PEG Smear Low	100/250 mL	MD1-100(250)-258
50% v/v PEG Smear Medium	100/250 mL	MD1-100(250)-259
50% v/v PEG Smear High	100/250 mL	MD1-100(250)-260
50% v/v PEG Smear Broad	100/250 mL	MD1-100(250)-261

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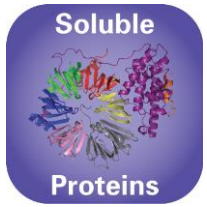
The BCS Screen Wells A1-D12 MD1-105



Well #	Conc.	Salt1	Conc.	Salt2	Conc.	Buffer	pH	Conc.	Precipitant1	Conc.	Precipitant2
A1					0.1 M	Sodium acetate	4.5	30 % v/v	PEG Smear Low		
A2					0.1 M	Phosphate/Citrate	5.5	30 % v/v	PEG Smear Low		
A3					0.1 M	MES	6.5	30 % v/v	PEG Smear Low		
A4					0.1 M	Sodium acetate	4.5	25 % v/v	PEG Smear Medium		
A5					0.1 M	Phosphate/Citrate	5.5	25 % v/v	PEG Smear Medium		
A6					0.1 M	MES	6.5	25 % v/v	PEG Smear Medium		
A7					0.1 M	Sodium acetate	4.5	20 % v/v	PEG Smear High		
A8					0.1 M	Phosphate/Citrate	5.5	20 % v/v	PEG Smear High		
A9					0.1 M	MES	6.5	20 % v/v	PEG Smear High		
A10					0.1 M	Sodium acetate	4.5	22 % v/v	PEG Smear Broad		
A11					0.1 M	Phosphate/Citrate	5.5	22 % v/v	PEG Smear Broad		
A12					0.1 M	MES	6.5	22 % v/v	PEG Smear Broad		
B1					0.1 M	HEPES	7.5	30 % v/v	PEG Smear Low		
B2					0.1 M	Tris	8.5	30 % v/v	PEG Smear Low		
B3					0.1 M	BICINE	9.3	30 % v/v	PEG Smear Low		
B4					0.1 M	HEPES	7.5	25 % v/v	PEG Smear Medium		
B5					0.1 M	Tris	8.5	25 % v/v	PEG Smear Medium		
B6					0.1 M	BICINE	9.3	25 % v/v	PEG Smear Medium		
B7					0.1 M	HEPES	7.5	20 % v/v	PEG Smear High		
B8					0.1 M	Tris	8.5	20 % v/v	PEG Smear High		
B9					0.1 M	BICINE	9.3	20 % v/v	PEG Smear High		
B10					0.1 M	HEPES	7.5	22 % v/v	PEG Smear Broad		
B11					0.1 M	Tris	8.5	22 % v/v	PEG Smear Broad		
B12					0.1 M	BICINE	9.3	22 % v/v	PEG Smear Broad		
C1								35 % v/v	PEG Smear Low		
C2	0.2 M	Ammonium acetate			0.1 M	Sodium acetate	4.6	28 % v/v	PEG Smear Low	5 % v/v	Ethylene glycol
C3	0.15 M	Sodium chloride						28 % v/v	PEG Smear Medium		
C4	0.2 M	Ammonium sulfate			0.1 M	Sodium cacodylate	5.5	25 % v/v	PEG Smear Medium		
C5	0.1 M	Sodium/potassium phosphate pH 5.5	0.1 M	Rubidium chloride	0.1 M	Sodium citrate	5.5	25 % v/v	PEG Smear Medium		
C6	0.2 M	Potassium chloride						22.5 % v/v	PEG Smear High		
C7	0.15 M	Ammonium acetate			0.1 M	Sodium citrate	5.0	15 % v/v	PEG Smear High		
C8	0.05 M	L-Arginine	0.05 M	L-Glutamic acid monosodium salt hydrate				28 % v/v	PEG Smear Broad	5 % v/v	Glycerol
C9	0.15 M	Magnesium acetate tetrahydrate			0.1 M	Sodium citrate	5.6	20 % v/v	PEG Smear Broad		
C10	0.2 M	Ammonium sulfate			0.1 M	Sodium acetate	4.6	25 % v/v	PEG Smear Broad		
C11	0.2 M	Potassium sodium tartrate tetrahydrate			0.1 M	MES	6.0	25 % v/v	PEG Smear Low		
C12	0.1 M	Calcium chloride dihydrate	0.1 M	Magnesium chloride hexahydrate	0.1 M	PIPES	7.0	22.5 % v/v	PEG Smear Medium		
D1	0.2 M	Ammonium nitrate			0.1 M	Sodium cacodylate	5.3	22.5 % v/v	PEG Smear Low		
D2					0.1 M	MES	6.5	22.5 % v/v	PEG Smear Low	10 % v/v	2-Propanol
D3	0.15 M	Ammonium nitrate			0.1 M	MES	6.0	20 % v/v	PEG Smear Medium	5 % v/v	Ethylene glycol
D4	0.2 M	Sodium formate			0.1 M	Sodium phosphate	6.2	20 % v/v	PEG Smear Medium	10 % v/v	Glycerol
D5	0.2 M	Lithium sulfate			0.1 M	ADA	6.5	30 % v/v	PEG Smear Medium		
D6	0.1 M	Potassium thiocyanate	0.1 M	Sodium bromide	0.1 M	MES	6.5	12 % v/v	PEG Smear High		
D7	0.2 M	Ammonium sulfate			0.1 M	ADA	6.5	18 % v/v	PEG Smear High		
D8	0.15 M	Calcium chloride dihydrate			0.1 M	MES	6.2	15 % v/v	PEG Smear Broad	5 % v/v	Glycerol
D9	5 % v/v	T-mate pH 7.0			0.1 M	Sodium cacodylate	5.3	15 % v/v	PEG Smear Broad	10 % v/v	Ethylene glycol
D10	0.2 M	Sodium chloride			0.1 M	Sodium phosphate	6.2	28 % v/v	PEG Smear Broad		
D11	0.1 M	Ammonium sulfate	0.05 M	Magnesium sulfate heptahydrate	0.1 M	Sodium citrate	5.5	22.5 % v/v	PEG Smear Medium		
D12	0.01 M	Cobalt(II) chloride hexahydrate	0.2 M	Magnesium chloride hexahydrate	0.1 M	Bis-Tris propane	8.0	22.5 % v/v	PEG Smear Medium	2 % v/v	Glycerol

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The BCS Screen Wells E1-H12 MD1-105

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Well #	Conc.	Salt1	Conc.	Salt2	Conc.	Buffer	pH	Conc.	Precipitant1	Conc.	Precipitant2
E1	0.08 M	Magnesium acetate tetrahydrate	0.02 M	Magnesium chloride hexahydrate	0.1 M	MES	6.5	25 % v/v	PEG Smear Low		
E2	0.1 M	Potassium chloride			0.1 M	HEPES	7.5	16 % v/v	PEG Smear Low	5 % v/v	Ethylene glycol
E3	0.1 M	Zinc acetate dihydrate	0.1 M	Zinc chloride	0.1 M	Bis-tris	7.5	20 % v/v	PEG Smear Medium		
E4	0.1 M	Magnesium chloride hexahydrate	0.1 M	Potassium chloride	0.1 M	PIPES	7.0	20 % v/v	PEG Smear Medium		
E5	0.05 M	Magnesium sulfate heptahydrate			0.1 M	HEPES	7.5	28 % v/v	PEG Smear Medium		
E6	0.1 M	Sodium/potassium phosphate pH 7.5			0.1 M	HEPES	7.5	15 % v/v	PEG Smear High	10 % v/v	Ethylene glycol
E7	0.1 M	Magnesium formate dihydrate	0.1 M	Rubidium chloride	0.1 M	PIPES	7.0	25 % v/v	PEG Smear High		
E8	0.2 M	Lithium sulfate			0.1 M	HEPES	7.2	25 % v/v	PEG Smear Broad		
E9	0.2 M	Ammonium nitrate			0.1 M	HEPES	7.5	20 % v/v	PEG Smear Broad		
E10	0.1 M	Magnesium chloride hexahydrate	0.1 M	Rubidium chloride	0.1 M	HEPES	7.5	30 % v/v	PEG Smear Broad		
E11	0.05 M	Magnesium chloride hexahydrate	0.05 M	Sodium citrate tribasic dihydrate	0.1 M	Bis-Tris propane	7.8	22.5 % v/v	PEG Smear High		
E12	7 % v/v	T-mate pH 7.0			0.1 M	BICINE	9.0	22.5 % v/v	PEG Smear High	10 % v/v	Ethylene glycol
F1	0.15 M	Sodium citrate tribasic dihydrate			0.1 M	HEPES	7.8	25 % v/v	PEG Smear Low		
F2	0.2 M	Sodium chloride			0.1 M	Tris	8.5	28 % v/v	PEG Smear Low	5 % v/v	Glycerol
F3	0.075 M	Sodium acetate trihydrate	0.15 M	Sodium chloride	0.1 M	Tris	8.0	15 % v/v	PEG Smear Medium		
F4	0.1 M	Sodium chloride	0.1 M	Sodium formate	0.1 M	Bis-Tris propane	8.5	25 % v/v	PEG Smear Medium		
F5	0.2 M	Ammonium sulfate	0.05 M	Magnesium sulfate heptahydrate	0.1 M	BICINE	9.0	20 % v/v	PEG Smear Medium		
F6	0.2 M	Ammonium nitrate			0.1 M	Bis-Tris propane	8.5	18 % v/v	PEG Smear High		
F7	0.2 M	Magnesium chloride hexahydrate			0.1 M	Tris	8.0	25 % v/v	PEG Smear High	10 % v/v	Glycerol
F8	0.15 M	Ammonium acetate	0.01 M	Calcium chloride dihydrate	0.1 M	Tris	8.5	28 % v/v	PEG Smear Broad		
F9					0.1 M	BICINE	9.0	25 % v/v	PEG Smear Broad	10 % v/v	2-Propanol
F10	0.2 M	Ammonium sulfate			0.1 M	Tris	8.0	20 % v/v	PEG Smear Broad		
F11	0.02 M	Magnesium sulfate heptahydrate	0.2 M	Potassium chloride	0.1 M	BICINE	8.8	22.5 % v/v	PEG Smear Broad		
F12	0.1 M	Potassium sodium tartrate tetrahydrate			0.1 M	Sodium cacodylate	5.5	22.5 % v/v	PEG Smear Broad	10 % v/v	Ethylene glycol
G1	0.01 M	Cobalt(II) chloride hexahydrate	0.1 M	Magnesium formate dihydrate	0.1 M	MES	6.2	14 % v/v	PEG Smear Low		
G2	0.15 M	Lithium sulfate	0.05 M	Magnesium chloride hexahydrate	0.1 M	Bis-Tris	6.8	25 % v/v	PEG Smear Low		
G3	0.2 M	Ammonium sulfate	0.01 M	Cadmium chloride hemi(pentahydrate)	0.1 M	HEPES	7.5	25 % v/v	PEG Smear Medium		
G4	0.1 M	Potassium chloride	0.1 M	Magnesium chloride hexahydrate				18 % v/v	PEG Smear Medium	10 % v/v	Ethylene glycol
G5	0.1 M	Magnesium acetate tetrahydrate			0.1 M	MES	6.5	12 % v/v	PEG Smear Medium	10 % v/v	Ethylene glycol
G6	0.1 M	Magnesium acetate tetrahydrate	0.1 M	Potassium chloride	0.1 M	MES	6.2	12 % v/v	PEG Smear High		
G7	0.04 M	Calcium chloride dihydrate	0.04 M	Sodium formate	0.1 M	PIPES	7.0	8 % v/v	PEG Smear High		
G8	0.075 M	Magnesium chloride hexahydrate	0.075 M	Sodium citrate tribasic dihydrate	0.1 M	Bis-Tris	6.0	18 % v/v	PEG Smear Broad		
G9	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium acetate trihydrate	0.1 M	Bis-Tris	6.5	15 % v/v	PEG Smear Broad		
G10	0.1 M	Ammonium sulfate	0.1 M	Sodium formate	0.1 M	HEPES	7.0	25 % v/v	PEG Smear Broad		
G11	0.2 M	Sodium/potassium phosphate pH 7.5			0.1 M	HEPES	7.5	22.5 % v/v	PEG Smear Medium	10 % v/v	Glycerol
G12	0.3 M	Sodium chloride	0.05 M	L-Arginine	0.1 M	Tris	7.5	22.5 % v/v	PEG Smear Broad	0.05 M	L-Glutamic acid monosodium salt hydrate
H1	0.04 M	Calcium chloride dihydrate	0.04 M	Sodium formate	0.1 M	Tris	8.0	25 % v/v	PEG Smear Low		
H2	0.1 M	Magnesium chloride hexahydrate	0.1 M	Rubidium chloride	0.1 M	PIPES	7.0	20 % v/v	PEG Smear Low		
H3	0.2 M	Magnesium chloride hexahydrate	10 % v/v	Ethylene glycol	0.1 M	HEPES	7.5	15 % v/v	PEG Smear Medium	5 % v/v	2-Propanol
H4	0.05 M	Ammonium acetate	0.15 M	Magnesium sulfate heptahydrate	0.1 M	HEPES	7.0	12 % v/v	PEG Smear Medium		
H5	7 % v/v	T-mate pH 7.0			0.1 M	HEPES	7.2	20 % v/v	PEG Smear Medium		
H6	0.1 M	Ammonium acetate	0.1 M	Zinc chloride	0.1 M	Bis-Tris	7.2	15 % v/v	PEG Smear High		
H7	0.15 M	Lithium sulfate	0.05 M	Magnesium chloride hexahydrate	0.1 M	HEPES	7.8	20 % v/v	PEG Smear High		
H8	0.1 M	Potassium thiocyanate	0.1 M	Sodium bromide	0.1 M	Tris	7.8	25 % v/v	PEG Smear Broad		
H9	0.05 M	Ammonium sulfate	0.05 M	Lithium sulfate	0.1 M	Bis-Tris propane	8.5	28 % v/v	PEG Smear Broad		
H10	0.2 M	Ammonium sulfate	0.01 M	Cadmium chloride hemi(pentahydrate)	0.1 M	PIPES	7.0	15 % v/v	PEG Smear Broad	10 % v/v	Ethylene glycol
H11	0.2 M	Lithium sulfate	0.05 M	Zinc acetate dihydrate	0.1 M	Bis-Tris	7.5	22.5 % v/v	PEG Smear Broad		
H12	0.075 M	Sodium bromide	0.05 M	Sodium fluoride	0.1 M	HEPES	7.8	22.5 % v/v	PEG Smear Broad	0.075 M	Sodium iodide